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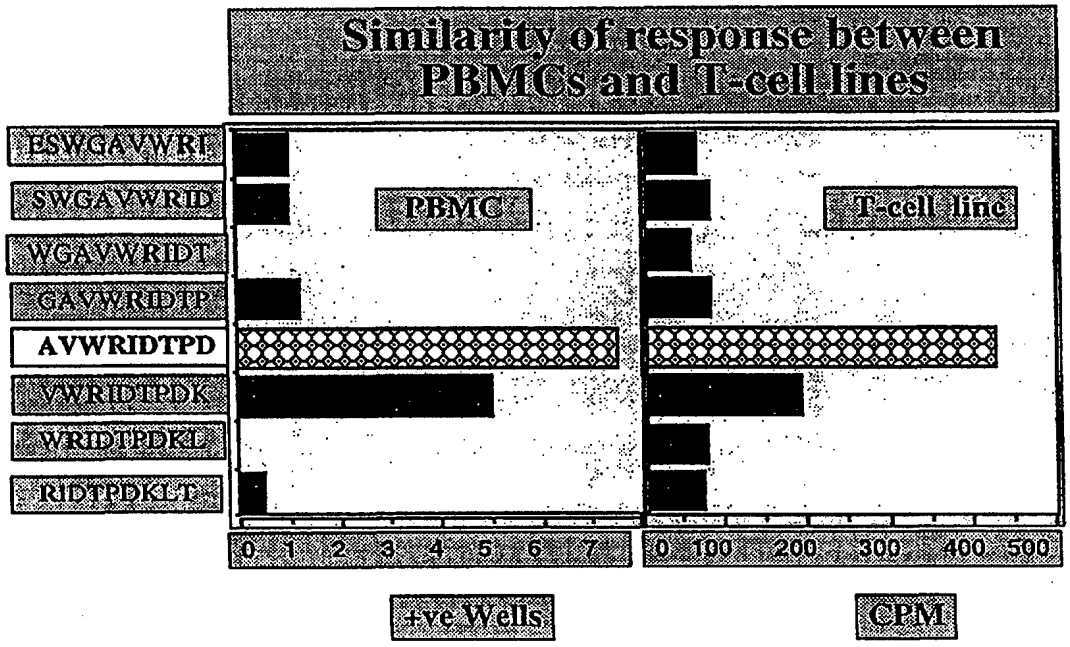

(57) Abstract

The present invention relates to the immunodominant T cell epitope of rye grass, its minimal sequence, its role in hayfever and its use in the manufacture of a medicament for the treatment or prophylaxis of hayfever. According to the present invention there is provided a polypeptide of nine amino acid residues including the six amino acid residues VXRIDT, and analogues and mimetics thereof. The polypeptide can be administered to provide a method for treatment or prophylaxis of allergies particularly of rye grass allergy or hayfever.

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T Cell Epitopes Of Rye Grass Pollen Allergen

The present invention relates to the immunodominant T cell epitope of rye grass, its minimal sequence, its role in hayfever and its use in the manufacture of a medicament for the treatment or prophylaxis of hayfever.

Amino acid residue symbols used hereafter are those identified by the IUPAC-IUB Biochemical Nomenclature Commission and they include all D or L amino acids or analogues and derivatives thereof. The symbol X represents an unidentified amino acid or analogue thereof.

One of the major causes of hayfever in Europe is the pollen of rye grass, *Lolium perenne*, which is a common allergen. Several proteins from the pollen of rye grass have been isolated and the complete amino acid sequences for three of these proteins (*Lol pI*, *Lol pII* and *Lol pIII*) have been published.

The major fraction of *Lolium perenne* (*L. perenne*) has been found to be *Lol pI*, a glycoprotein of 240 amino acid residues, and most hayfever sufferers in Europe sensitive to grass pollen (about 80%) possess specific IgE antibodies to this protein. The complete sequence of *Lol pI* has been published and it has

been suggested that there is a polypeptide sequence of 14 consecutive amino acid residues which includes the immunogenic determinant of *Lol pI*. (Bungy *et al*. Clin Exp Immunol 1993, 94, 111-116). When tested *in vivo* by intradermal skin testing such a polypeptide could show typical delayed type hypersensitivity (DTH) reactions after 24-48 hours in atopic rye grass-sensitive patients. Current guidelines suggest that allergic symptoms (for example those of hayfever) limited to the nose should be treated by immunotherapy, especially where hayfever symptoms are not controlled by conventional medication. It is also suggested that patients with pollen asthma should be excluded from such treatment because of potential side effects.

Immunotherapy using native allergen to reduce the sensitivity of patients has the disadvantage that all sensitising agents have the potential to induce severe allergic reactions such as bronchospasm, anaphylaxis and even death.

Clinical hyposensitisation for hayfever using an incremental regime of injection of whole extract of allergens is not curative, although symptoms are ameliorated. However, whole extracts contain an IgE binding site which can result in anaphylactic shock and even death. Although pharmacological

treatment can relieve mild symptoms, there is as yet no immunological "cure" for the severe cases, especially those with asthma.

One approach to overcome these problems has been the development of modified allergens for use in vaccines which might first be more effective, and second, much safer.

The availability of a safe and effective peptide vaccine would allow treatment to be given to a very important group of patients.

Studies of the desensitisation effect of an enzyme digest of pollen comprising a T cell epitope suggest that an allergic response to pollen may be reduced in both animal and man (Bungy *et al* supra). There is potential clinical benefit if an immuno dominant T cell epitope could be used to induce anergy or specific T cell unresponsiveness locally or systemically in patients allergic to a major allergen such as rye or other grasses.

Hayfever symptoms are brought about through specific lymphocyte activation leading to the release of vasoactive amine mediators (e.g. histamine) from cells into extracellular space. The role

of IgE antibodies in mediating allergic reactions (for example, those of hayfever) is well known.

The ability to trigger T cells could be used in the production of antibodies to a B cell epitope by combination of this B cell epitope with a T cell epitope. Such antibodies could be used to block the IgE mediated response to allergen (for example blocking of binding of IgE to cells).

IgE comprises two light polypeptide chains and two heavy polypeptide chains linked by disulphide bonds. The heavy chains have one variable domain (V_H) and four constant domains (C_H1, C_H2, C_H3 and C_H4 also known as $C_\varepsilon1$, $C_\varepsilon2$, $C_\varepsilon3$ and $C_\varepsilon4$). A region of IgE which comprises part of the heavy chains is known as the Fc region.

As disclosed by Stanworth *et al* (WO 90/15778, the content of which is incorporated herein by reference), investigations have been carried out in order to define the nature of the "effector" site within the IgE Fc region which is thought to provide the immunological signal resulting in histamine release. Structure-activity studies have been carried out on model histamine-releasing polypeptides and this has indicated that a cluster of basic amino acids are essential for the direct triggering of

histamine release. Furthermore, the presence of neighbouring hydrophobic residues and the amidation of their C-terminal carboxylic acid residues has been found to enhance triggering of histamine release.

Based on the above observations, the Fc region of human IgE, the structure of which has been elucidated, was examined for amino acid residue sequences which fulfilled such criteria. The amino acid residue sequence spanning residues 496-506 of the $C_{\varepsilon}4$ domain: RKTKGSGFFVF [SEQ ID:1] seemed the most likely to meet these criteria.

WO 90/15878 describes certain immunoactive peptides and antibodies and their use in allergy treatment by prevention of the triggering of the release of histamine. The Stanworth application described an immunogen comprising a residue of a histamine-releasing peptide comprising a cationic N-terminal head and a hydrophobic C-terminal tail, together with a residue capable of eliciting antibodies against said peptide whilst inhibiting histamine-release by said peptide is useful in anti-allergy treatment. Preferably the histamine-releasing peptide is of formula: KTKGSGFFVF [SEQ ID:2], optionally amidated at the C-terminal. Antibodies to the histamine-releasing peptide are used for passive immunisation.

According to the present invention there is provided the minimal T cell epitope of rye grass, which is an immunodominant 9-mer polypeptide active in stimulating T cells from atopic patients with hayfever. T cells specifically sensitive to any or some of the individual proteins *Lol pI*, *Lol pII* and *Lol pIII* may be stimulated by this immunodominant 9-mer.

According to the present invention there is provided a polypeptide having nine amino acid residues and including the six amino acid sequence VXRIDT [SEQ ID:3]. The sequence VXRIDT may be referred to as the T cell epitope motif.

Also according to the invention there is provided a polypeptide having nine amino acid residues of the sequence XVXRIDTXX, [SEQ ID:4].

Also according to the invention there is provided a polypeptide having a sequence of nine amino acid residues of the sequence AVWRIDTPD [SEQ ID:5] or VWRIDTPDK [SEQ ID:6] which has 0, 1 or 2 of its amino acid residues substituted for any other amino acid residue.

The polypeptides of the present invention having nine amino acid residues may be complexed to a hapten (e.g. phosphorylcholine

or 2,4 dinitrophenyl) or a pharmacologically acceptable carrier or both; complexed either directly or linked indirectly by way of a spacer such as a polypeptide or a hydrocarbon or co-administered. Accordingly, there is provided a conjugate comprising a pharmacologically acceptable carrier and a polypeptide having nine amino acid residues including the T cell epitope motif VXRIDT, or nine amino acid residues of the sequence XVXRIDTXX, or the sequence AVWRIDTPD or VWRIDTPDK having 0, 1 or 2 of its amino acid residues substituted for any other amino acid residue. Furthermore, there is also provided by the present invention a ligand comprising an antibody domain specific for the nine amino acid polypeptide of the present invention. This antibody domain may be mono- or polyclonal or antigen binding fragments thereof.

The immunodominant 9-mer polypeptide of the invention may be used to induce T cell activation. This, in turn, could lead to T cell anergy, diminution or lack of responsiveness to an antigen.

In a second aspect, the present invention provides an immunogen comprising a residue of a histamine releasing peptide comprising a cationic N-terminal head and a hydrophobic C-terminal tail, together with a residue capable of eliciting antibodies against

this peptide, together with a 9-mer polypeptide as previously described.

A purpose of this second aspect of the present invention is to induce production of antibodies to the immunogen in individuals sensitive to rye grass pollen.

The present invention can include an immunogen comprising a residue of a histamine-releasing peptide which has a cationic N-terminal head comprising the sequence KTK, and a hydrophobic C-terminal tail comprising the sequence FF. The C-terminal may be blocked by amidation. The N-terminal head may be separated from the C-terminal tail by from two to six predominantly non-polar and non-hydrophobic amino acid residues (for example GSG).

The present invention can provide an immunogen including the peptide sequence KTKGSGFFVF, together with a polypeptide having 9 amino acid residues as described above, wherein the 9-mer polypeptide is complexed directly to the immunogen or is indirectly linked e.g. by way of a spacer such as a polypeptide or hydrocarbon or the 9-mer polypeptide is co-administered with the immunogen.

Preferably the present invention provides an immunogen which

includes both a residue of a histamine-releasing polypeptide having the sequence KTKGSGFFVF, and a T cell epitope polypeptide having the sequence AVWRIDTPD, either complexed directly or linked indirectly e.g. by way of a spacer such as a polypeptide or hydrocarbon or the 9-mer polypeptide is co-administered with the immunogen. Preferably the C-terminal of the immunogen is blocked by amidation.

In a third aspect, the present invention provides a method of using the above compounds and immunogens of the present invention in the manufacture of a medicament for the treatment or prophylaxis of allergies particularly of rye grass allergy or hayfever.

In a fourth aspect, the present invention provides a method for treatment or prophylaxis of allergies particularly of rye grass allergy or hayfever, by administration of an effective amount of the above compounds or immunogens.

Medicaments made according to the invention may be administered to patients by any of the administration methods already known to those skilled in the art.

In a fifth aspect, the present invention provides an assay for

sensitivity to rye grass allergens and hence to determination of hayfever sufferance. In such an assay peripheral blood mononuclear cells (PBMCs) may be cultured in the presence of the immunodominant 9-mer T cell epitope and lymphocyte activation may be measured using thymidine incorporation as determinant. Cells from subjects allergic to any of the main allergens of rye grass would be expected to react.

In a sixth aspect, the present invention provides an assay for specific T cell populations sensitive to rye grass allergen, such as *Lol pI*, by testing for reaction to the 9-mer described above, selecting reactive T cells and forming therefrom T cell lines, and cloning therefrom a T cell clone having specificity for the 9-mer.

The present invention will be further illustrated with reference to the drawings of which:

Figure 1 shows the peripheral blood mononuclear cell response of an atopic patient (code name AS3) to *Lol pI*;

Figure 2 shows PBMC responses to 20 peptide pools;

Figure 3 shows the decoding of the immunodominant Pool 17;

Figure 4 shows dose response curves for peptides of Pool 17;

Figure 5 shows PBMC responses of atopic patient AS3 to 9 mer peptides;

Figure 6 shows T cell line specificity for *Lol pI*;

Figure 7 shows a comparison of T cell line and PBMC responses to 9-mer peptides;

Figure 8 shows the results of Lysine substitution when each amino acid residue of the 14 amino acid polypeptide thought to include the T cell epitope of *Lol pI* was individually substituted for Lysine; and

Figure 9 shows the results of Glycine substitution when each amino acid residue of the 14 amino acid residue polypeptide thought to include the T cell epitope of *Lol pI* was individually substituted for Glycine.

Rye grass *(Lolium perenne)* pollen extract was supplied by Dome Laboratories (Stoke Court, U.K.) and subjected to 33% ammonium sulphate precipitation. The supernatant was dialysed against phosphate buffer saline (PBS). The dialysed sample was injected

on to FPLC (fast protein liquid chromatography) (Pharmacia, LKB, Sweden) for protein separation on a gel filtration column Superose 12, and 0.3 ml fractions were collected. Sodium dodecyl sulphate (SDS) polyacrylamide-gel electrophoresis (PAGE) was performed on a vertical slab-gel apparatus (Bio-rad, Richmond, CA). 15% gradient acrylamide gels were employed in this study and run at 180 V for 12 hours. The apparent molecular mass of the allergens was estimated by using prestained protein standards.

The iso-allergen clone 5A of *Lol pI*, consists of 240 amino acids. A set of 115 overlapping, 12-mer peptides spanning the entire length of the iso-allergen clone 5A of the *Lol pI* was synthesized on polyethylene rods (pins) using the multi-pin technique which enables the rapid mapping of T cell determinants. All of the peptides were acetylated at the N terminus and, as a result of cleavage from the pins, had a C-terminal diketopiperazine (DKP) group.

A ß-alanine residue was incorporated to space the defined sequence from the DKP group. The peptides were released from the solid phase according to the method of Maeji *et al.* using 0.1M phosphate buffer (pH 7) for 4 hours at room temperature. Sets of six continuous peptides were pooled, with the exception

of Pools-1 and 15 (5 peptides) and Pool-20 (3 peptides). Therefore, 20 peptide pools were used for epitope mapping. The purity of selected peptides including all peptides in Pool-17, was established by reverse phase HPLC analysis and the amino acid composition was checked by amino acid analysis.

100 ml of blood from each subject was collected in preservative free heparin. Heparinized venous blood was diluted 1:1 with RPMI 1640 and layered onto Lymphoprep (Nycomed, Oslo, Norway). The gradients were centrifuged at 400g for 25 minutes at room temperature. Mononuclear cells were harvested and washed twice in RPMI 1640 (Gibco, Grand Island, NY, USA) and then resuspended at a concentration of $2x10^6$ cells/ml in RPMI supplemented with 10% human AB-serum (ICN Flow, Bucks, U.K.), 2mM 1-glutamine and 20μg/ml gentamicin (Gibco, Grand Island, NY, USA). Cells were cultured at a concentration of 2×10^5 per well in 0.2 ml of complete medium in U-bottomed 96-well microtiter plates.

Purified *Lol pI* or *Lolium perenne* extract (LPE) was added at a range of concentrations and PPD or Con-A was used as a positive control. Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 in air. After 5-6 days, 0.4μCi/well [methyl-^{3}H] TdR (Amersham, U.K.) was added and 6-18 hours later the cells were harvested onto Titertek glass-fibre

paper (ICN Flow, Bucks, U.K.). ^{3}H-thymidine incorporation into DNA was measured by liquid scintillation (National Diagnostics, Atlanta, USA) counting (Minaxi Tri-Carb 4000 Series, Packard).

Since the precursor frequency of T cells specific to any one peptide is very low, 12-24 replicate cultures were used for each of the 20 peptide pools at a final concentration of 1 μg/ml of each peptide. Twenty pools of peptides were screened, each pool comprising 6 overlapping peptides (with the exceptions as noted above) with successive peptides differing by only two amino acids at their starting (N-terminal) residue. The thymidine incorporation of each replicate was measured separately.

Purification of rye grass extract by FPLC gave two peaks (Peak 1 and 2). Electrophoresis of a *L. perenne* extract under reducing conditions followed by protein staining showed that the molecular weight of peak 1 was approximately 31 kDa and its location was equivalent to highly purified *Lol pI*. The other peak was identified as a mixture of *Lol pII* and *Lol pIII* with a molecular weight of 11 kDa.

Human peripheral blood mononuclear cells (PBMC) proliferative responses to the major purified fraction of *L. perenne (Lol pI)* were found (Fig.1). This dose ranging experiment shows the

typical bell-shaped dose response curve of lymphocyte activation seen with allergen and antigen. The peak incorporation of tritiated thymidine is seen at 10μg/ml of purified *Lol pI*. This is evidence of lymphocyte reactivity to the major fraction of Rye grass protein in atopic subjects. Atopics responded to both *Lol pI* and LPE as might be expected. Non-atopics did not respond to *Lol pI* but some did show minor stimulation with LPE.

The presence of T cell epitopes in the 20 pools of peptides was investigated by *in vitro* proliferation tests on PBMC of six rye grass-sensitive patients and seven non-atopic control subjects. Whilst five out of the six patients responded to Pool 17, patient B reacted to Pool 15 and 19. In addition, patient E responded to Pool 6 and patient F also responded to Pool 13. Pool 17 clearly contained the immunodominant epitope. Non-atopic subjects did not respond to any of the 20 peptide pools. There was no class II MHC (major histocompatibility complex) Dr restriction in the atopic subjects.

The number of wells containing peripheral blood mononuclear cells giving positive proliferative responses to 20 different peptide pools spanning the entire length of the *Lol pI* is shown in Fig. 2. Each pool was composed of 6 (except 1, 15 and 20) overlapping 12-mer peptides offset by 2 residues, and thus

spanned 22 residues of the protein sequence, and was tested against 12 identical cultures from each patient. These data identify Pool 17 as containing the major epitope of rye grass. The cut-off for significance in terms of number of wells responding is defined by statistical methods based on the Poisson distribution. In this instance, Pool 17 is statistically different from all other pools tested. Further data from other atopic subjects confirmed the immunodominance of Pool 17. As is mentioned above, Pool 17 is statistically different from all the other pools tested. Further data from other atopic subjects confirmed the immunodominance of Pool 17. As is mentioned above, Pool 17 consists of 6 overlapping peptides covering a span of 22 amino acids.

Having identified Pool 17 as the immunodominant pool, each of the peptides it contained was tested separately. Experiments showed that peptides 3 and 4 of Pool 17 were the main stimulatory peptides in vitro (Fig. 3). The active polypeptides of *Lol pI* (3) are ^{139}WGAVWRIDTPDK^{204} [SEQ ID:9] and (4) ^{195}AVWRIDTPDKLT^{206} [SEQ ID:10] and are each as active in lymphoproliferative assays as is the whole pool.

When large numbers of replicates are tested with any given antigen, it is clear that the positive wells are not normally

distributed. This is because of the random distribution of the low numbers of antigen specific T cells among the replicate wells. For this reason we have not treated the proliferation data using statistical methods based on normally distributed data but on the Poisson model. The non-responder wells are normally distributed and we have therefore taken a mean +/- 3SD as the cut-off to score wells as either positive or negative. Any well with counts above the means and three standard deviations is considered as positive.

Of Pool 17, only 2 out of the 6 peptides have stimulator activity. This activity or lack of activity of different Pool 17 peptides could have been dose related and thus dose response curves were generated.

PBMCs were incubated with a range of concentration of a) Pool 17, b) peptide 4 of Pool 17 and c) Pool 8 (a non-stimulatory peptide pool). Cells were harvested after 6 days in culture.

The dose response of peptide 4 of Pool 17 is parallel to whole Pool 17 whilst the non-stimulatory pool (Pool 8) is still ineffective in activating lymphocytes at 100 times the concentration of the peptide.

Thus, finding that Pool 17 is dominant is not due to an effect of final concentration because the non-stimulatory pool (Pool 8) was still ineffective at high concentrations.

Eight overlapping 9-mer peptides offset by one residue were screened using PBMCs from the atopic patient (AS3). The presence of minimal T cell epitopes was investigated by in vitro proliferation tests against single peptides. The thymidine incorporation of each replicate was measured separately.

Figure 5 shows the percentage of wells containing peripheral blood mononuclear cells from a rye grass sensitive patient (AS3) giving positive proliferative responses to single peptides of the 9-mer peptides spanning the entire length of the immunodominant epitope of the *Lol pI*.

Surprisingly, as shown in Fig. 5, the 9 amino acid residue polypeptide of sequence (5) AVWRIDTPD is found to be the most active of any 9, 10, 11, 12, 13 or 14 amino acid residue polypeptide spanning the length of the two continuous immunodominant polypeptides. The 9 amino acid residue polypeptide of sequence (6) VWRIDTPDK was also found to be active. More surprisingly, six amino acid residues were found to be the most important in determining whether the polypeptide

is immunodominant.

To supplement the data obtained from PBMC responses to 9-mer peptides, T cell line responses to 9-mer peptides were investigated.

Lol pI-specific T cell lines were obtain as follows: 0.5 x 10^6 PBMCs per ml from patient AS3 who reactive to the immunodominant T cell epitope were stimulated with *Lol pI* (10μg/ml) in PRMI 1640 supplemented with 2mM L-glutamine, 5 x 10^{-5}M 2-mercaptoethanol, 20μg/ml gentamicin, and 10% human AB serum in 24-well flat-bottomed culture plates for 5 days. Subsequently, human recombinant IL-2 (hrIL-2) (25 units/ml) was added and kept in culture for an additional 7 days. Viable T cell blasts were then separated by a Ficoll-Hypaque density gradient and *Lol pI* specificity of T cell line was assessed. The specificity of the T cell lines produced by stimulation with IL-2 and *Lol pI* was checked. 5 x 10^4 T cell line cells were incubated in duplicate 200 μl cultures in the presence of 5 x 10^4 irradiated autologous antigen presenting cells (APCs) plus 1, 5 and 10 μg/ml of *Lol pI* in 96-well round-bottomed microtiter plates for 3 days at 37°C, in a humidified atmosphere of 5% CO_2, then pulsed overnight with 0.4 μCi/well 3H-thymidine. Radionuclide uptake was

measured by scintillation counting. As shown in Fig. 6, using a dose response of *Lol pI* from 1 μg/ml to 10 μg/ml it is clear that this T cell line is specific for *Lol pI*. T cell line (T) plus irradiated APC* (A) gives low background counts in comparison.

5 x 10^4 T cell line cells were incubated in duplicate 200 μl cultures in the presence of 5 x 10^4 irradiated autologous APCs plus 7.5 μg/well of individual single peptide of the 9-mers in 96-well round-bottomed microtiter plates for 3 days at 37°C, in a humidified atmosphere of 5% CO_2, then pulsed overnight with 0.4 μCi/well 3H-thymidine. Radionuclide uptake was measured by scintillation counting. Two peptides (5) and (6) are clearly more active in stimulating T cell lines from an atopic donor than other peptides.

A comparison of PBMC and T cell line responses (Fig. 7) shows that the T cell line and PBMC respond similarly.

Experimentation on the previously known 14-mer peptides was carried out to attempt to identify parts of the T cell epitope minimal sequence which could be substituted with other amino acids. Results are shown in Fig. 8 for a series of 14-mer peptides having a single lysine (K) substitution and in Fig. 9

for a series having a single glycine (G) substitution. Substitution at a point which causes loss or reduction in activity indicates that the amino acid normally at that position is important or essential. In contrast certain substitutions at certain points have little or no effect on response. From this information it has been deduced that the nine amino acid residue polypeptide including the T cell epitope motif VXRIDT, or nine amino acid residues having the sequence XVXRIDTXX, or the sequence AVWRIDTPD or VWRIDTPDK wherein 0, 1 or 2 of the amino acid residues is substituted for any other amino acid residue is the common immunodominant T cell epitope of rye grass. The existence of a common epitope motif in rye grass was not obvious and its identification is of considerable clinical significance. In other areas of plant allergy (e.g. ragweed allergy) it has not been possible so far to characterise a common epitope to all of the various major allergy inducing proteins.

It will be apparent to those skilled in the art of immunology that the surprising characterisation of a T cell epitope motif and an immunodominant T cell epitope for rye grass for the first time allows the possibility of a previously impossible detailed analysis of the molecular environment of the immune response to rye grass allergen. In particular the motif and epitope can be

modelled to determine the shape and configuration of the polypeptides, the spatial distribution of charges, the level of steric constraint on the molecules etc. so as to determine the relationship between structure and activity. With such information it is foreseen that those skilled in the art could form analogs or mimetics of the motif and epitope, which would exhibit essentially the same or similar functional activity whilst differing in chemical formula. For example some or all amino acid residues could be replaced by equivalent non-amino-acid moieties. It is intended that the present invention should cover such functionally active analogue or mimetic T cell epitope or motif equivalents.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Arg Leu Thr Lys Gly Ser Gly Phe Phe Val Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Lys Thr Lys Gly Ser Gly Phe Phe Val Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Val Xaa Arg Ile Asp Thr
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Xaa Val Xaa Arg Ile Asp Thr Xaa Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ala Val Trp Arg Ile Asp Thr Pro Asp
1 5

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Val Trp Arg Ile Asp Thr Pro Asp Lys
1 5

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Leu Lys Glu Ser Trp Gly Ala Val Trp Arg Ile Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Glu Ser Trp Gly Ala Val Trp Arg Ile Asp Thr Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Trp Gly Ala Val Trp Arg Ile Asp Thr Pro Asp Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Ala Val Trp Arg Ile Asp Thr Pro Asp Lys Leu Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Trp Arg Ile Asp Thr Pro Asp Lys Leu Thr Gly Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Ile Asp Thr Pro Asp Lys Leu Thr Gly Pro Phe Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Glu Ser Trp Gly Ala Val Trp Arg Ile
1 5

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Ser Trp Gly Ala Val Trp Arg Ile Asp
1 5

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Trp Gly Ala Val Trp Arg Ile Asp Thr
1 5

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Gly Ala Val Trp Arg Ile Asp Thr Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Trp Arg Ile Asp Thr Pro Asp Lys Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Arg Ile Asp Thr Pro Asp Lys Leu Thr
1 5

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION:1..14
(D) OTHER INFORMATION:/note= "EACH RESIDUE IS INDIVIDUALY SUBSTITUTED WITH Lys TO GIVE 14 DIFFERENT SEQUENCES"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Trp Asp Ala Val Trp Arg Ile Asp Thr Pro Asp Lys Leu Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION:1..14
(D) OTHER INFORMATION:/note= "EACH RESIDUE IS INDIVIDUALLY SUBSTITUTED WITH Gly TO GIVE 14 DIFFERENT SEQUENCES
"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Trp Gly Ala Val Trp Arg Ile Asp Thr Pro Asp Lys Leu Thr
1 5 10

Claims

1. A polypeptide of nine amino acid residues including the six amino acid residue VXRIDT, and analogues and mimetics thereof.

2. A polypeptide according to claim 1, having the amino acid residue sequence XVXRIDTXX.

3. A polypeptide having the amino acid residue sequence AVWRIDTPD having 0, 1 or 2 of its amino acid residues substituted for any other amino acid residue, and analogues and mimetics thereof.

4. A polypeptide having the amino acid residue sequence VWRIDTPDK having 0, 1 or 2 of its amino acid residues substituted for any other amino acid residue, and analogues and mimetics thereof.

5. A polypeptide having the amino acid residue sequence according to any of claims 1 to 4, together with a polypeptide having the amino acid residue sequence KTKGSGFFVF either complexed directly or linked indirectly by way of a spacer such as a polypeptide or a hydrocarbon, or co-administered.

6. An immunogen comprising a residue of a histamine releasing peptide comprising a cationic N-terminal head and a hydrophobic C-terminal tail, together with a residue capable of eliciting antibodies against this peptide, together with a 9-mer polypeptide according to any of claims 1 to 4.

7. An immunogen comprising a residue of a histamine-releasing peptide which has a cationic N-terminal head comprising the sequence KTK,and a hydrophobic C-terminal tail comprising the sequence FF, the N-terminal head being separated from the C-terminal tail by from two to six predominantly non-polar and non-hydrophobic amino acid residues, together with a 9-mer polypeptide according to any of claims 1 to 4.

8. An immunogen according to claim 7, wherein the C-terminal is blocked by amidation.

9. An immunogen according to claim 7 or 8, wherein the sequence GSG separates the N- from the C-terminal.

10. A polypeptide according to any of claims 1 to 4, complexed to a hapten or a pharmacologically acceptable carrier or both; complexed either directly or linked indirectly by way

of a spacer such as a polypeptide or a hydrocarbon or co-administered.

11. Use of polypeptides and immunogens according to any of claims 1 to 10, in the manufacture of a medicament for the treatment or prophylaxis of allergies particularly of rye grass allergy or hayfever.

12. A method for treatment or prophylaxis of allergies particularly of rye grass allergy or hayfever, which comprises administration of an effective amount of a polypeptide or immunogen according to any of claims 1 to 10.

13. An assay for sensitivity to rye grass allergens for determination of hayfever sufferance, in which assay peripheral blood mononuclear cells (PBMCs) are cultured in the presence of a polypeptide according to any of claims 1 to 4, and lymphocyte activation is measured using thymidine incorporation as determinant.

14. An assay for specific T cell populations sensitive to rye grass allergen, such as *Lol pI*, which comprises testing T cells for reaction to the 9-mer polypeptide of any of claims 1 to 4, selecting reactive T cells and forming therefrom T cell

lines, and cloning therefrom a T cell clone or clones having specificity for the 9-mer.

Figure 1

PBMC responses to Lol p1 (AS3)

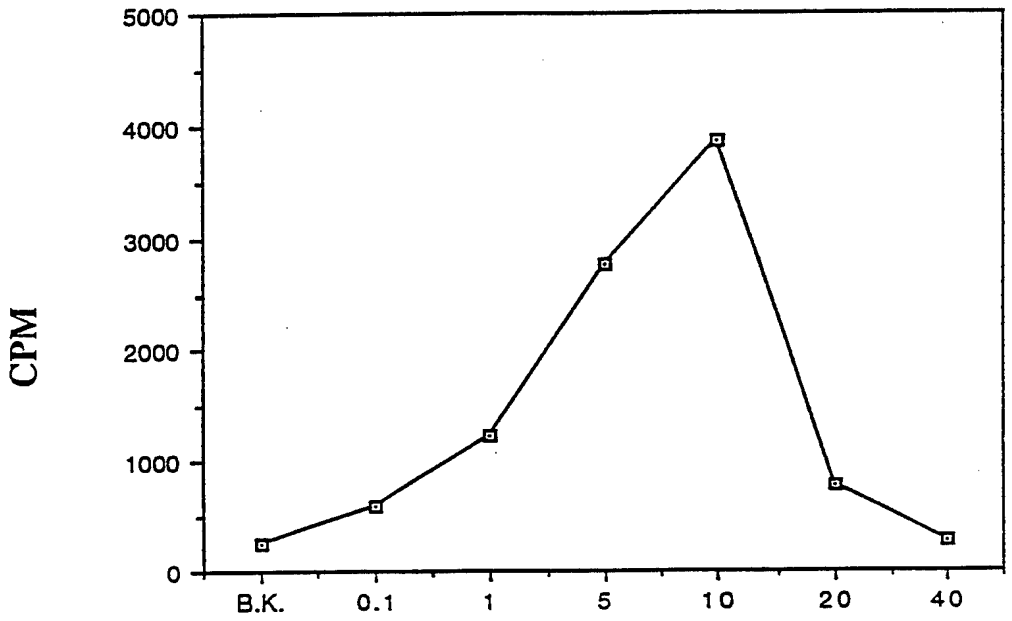


Figure 2

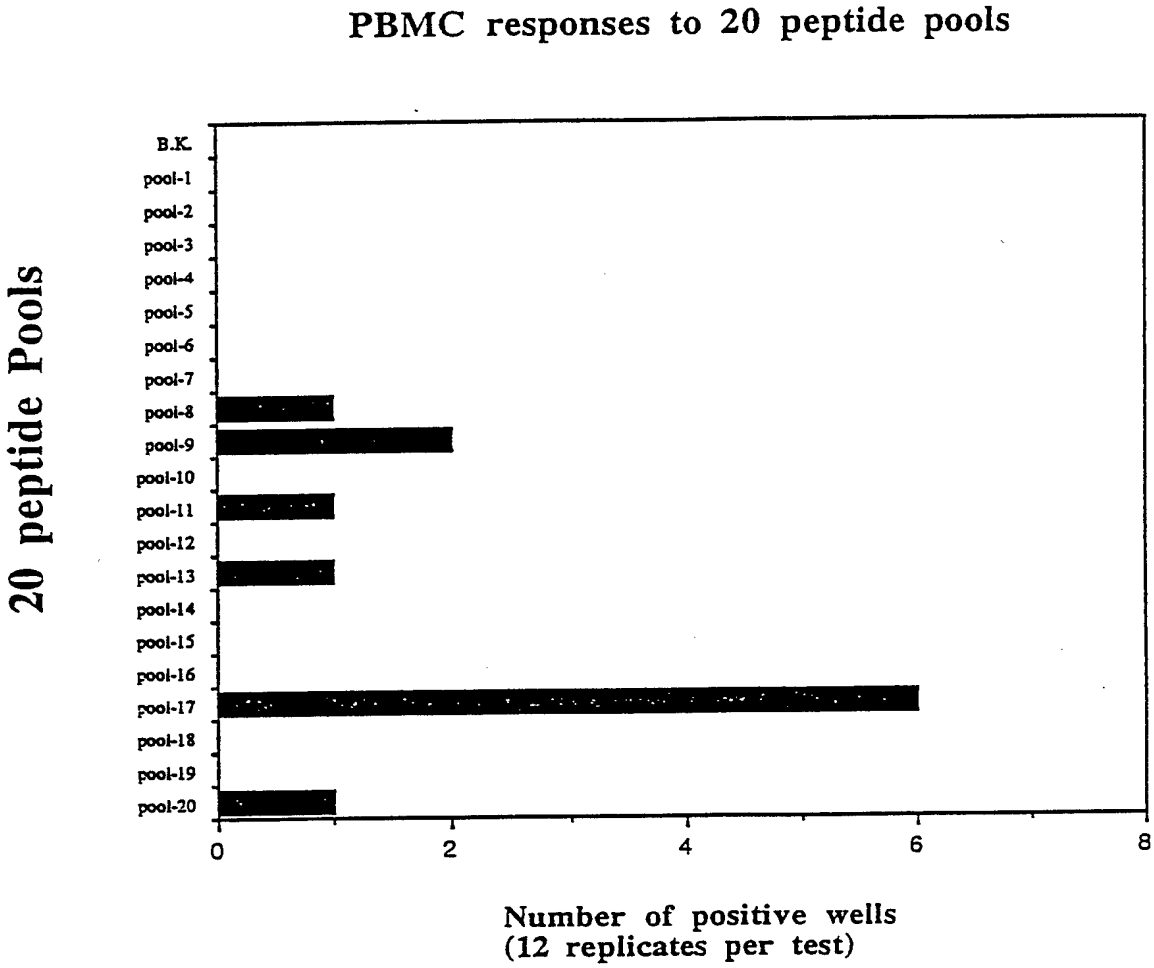

PBMC responses to 20 peptide pools
20 peptide Pools
B.K.
pool-1
pool-2
pool-3
pool-4
pool-5
pool-6
pool-7
pool-8
pool-9
pool-10
pool-11
pool-12
pool-13
pool-14
pool-15
pool-16
pool-17
pool-18
pool-19
pool-20
0
2
4
6
8
Number of positive wells
(12 replicates per test)

Figure 3

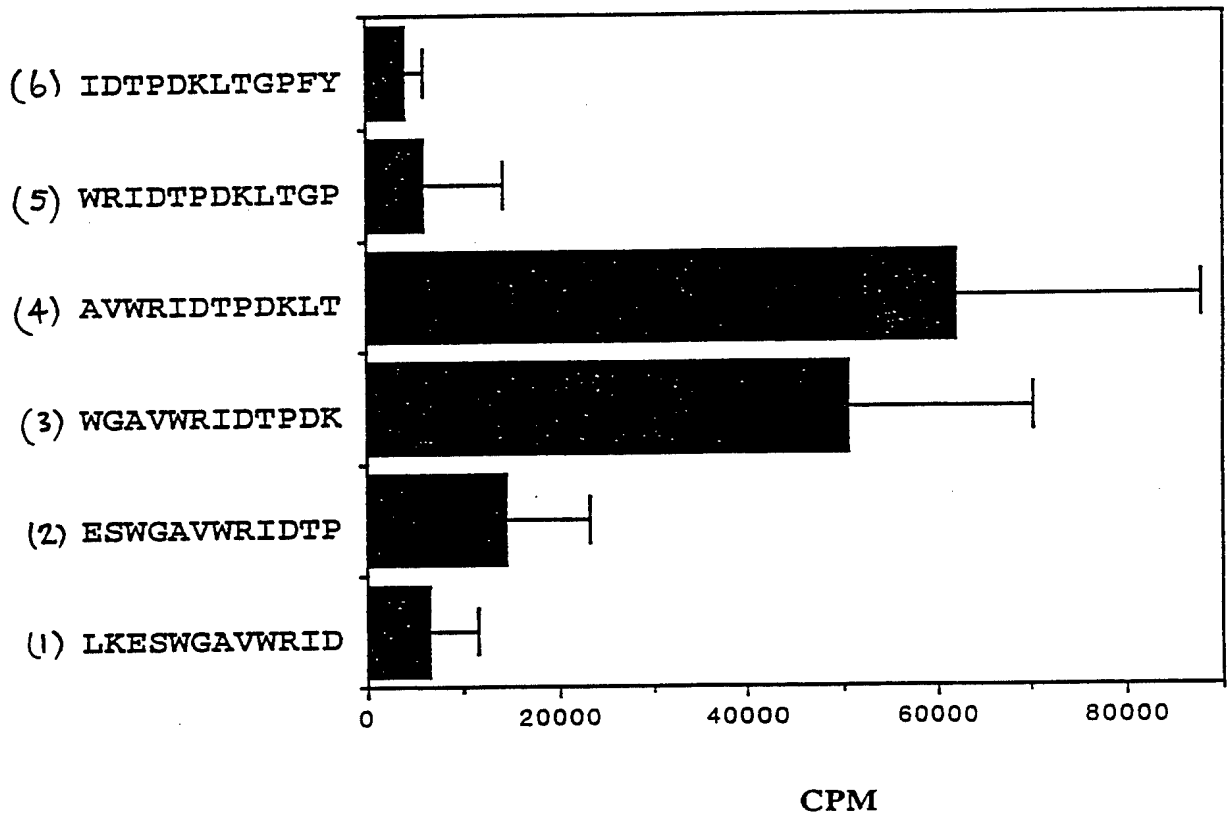

Decoding of the immunodominant pool (AS3)
(6) IDTPDKLTGPFY
(5) WRIDTPDKLTGP
(4) AVWRIDTPDKLT
(3) WGAVWRIDTPDK
(2) ESWGAVWRIDTP
(1) LKESWGAVWRID
0
20000
40000
60000
80000
CPM

Figure 4

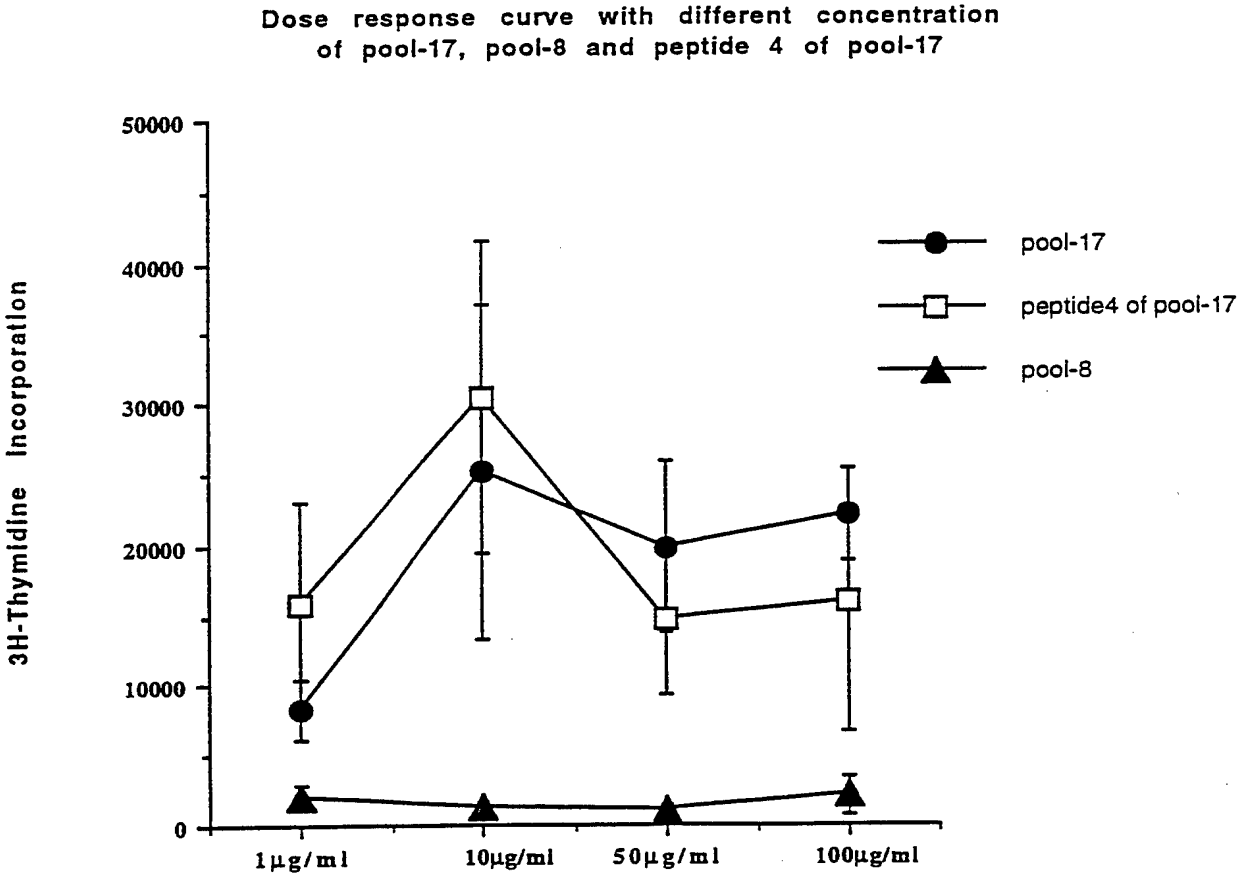

Dose response curve with different concentration of pool-17, pool-8 and peptide 4 of pool-17
3H-Thymidine Incorporation
50000
40000
30000
20000
10000
0
1µg/ml
10µg/ml
50µg/ml
100µg/ml
pool-17
peptide4 of pool-17
pool-8

Figure 5

PBMC responses to 9mer peptides
(AS3)

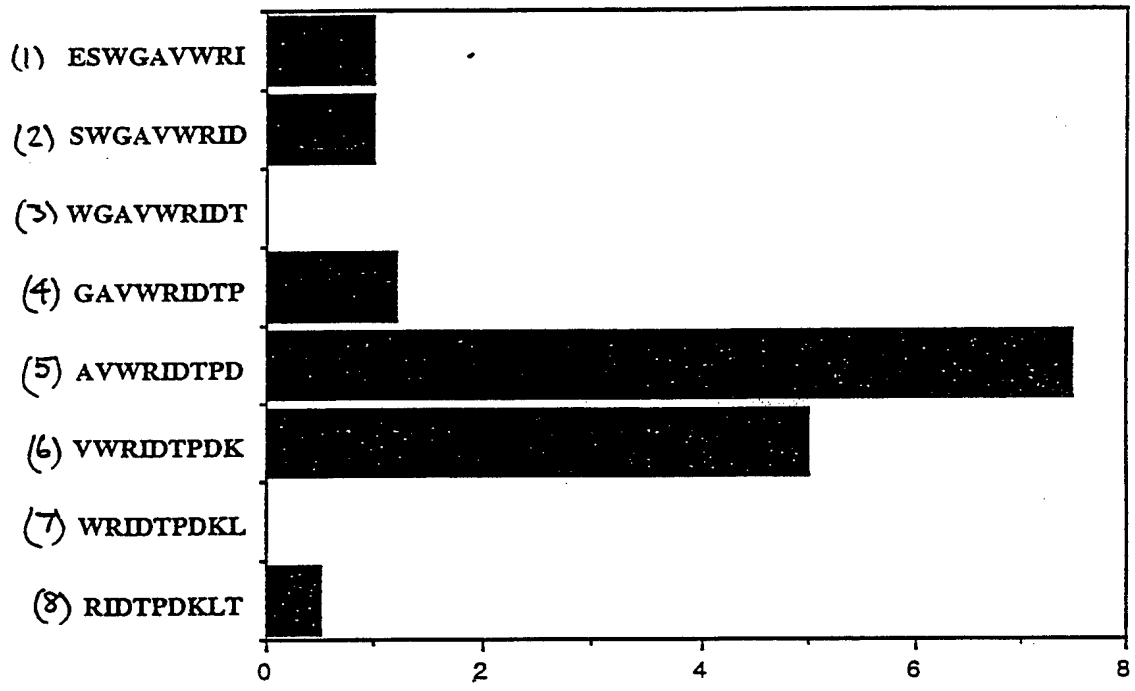
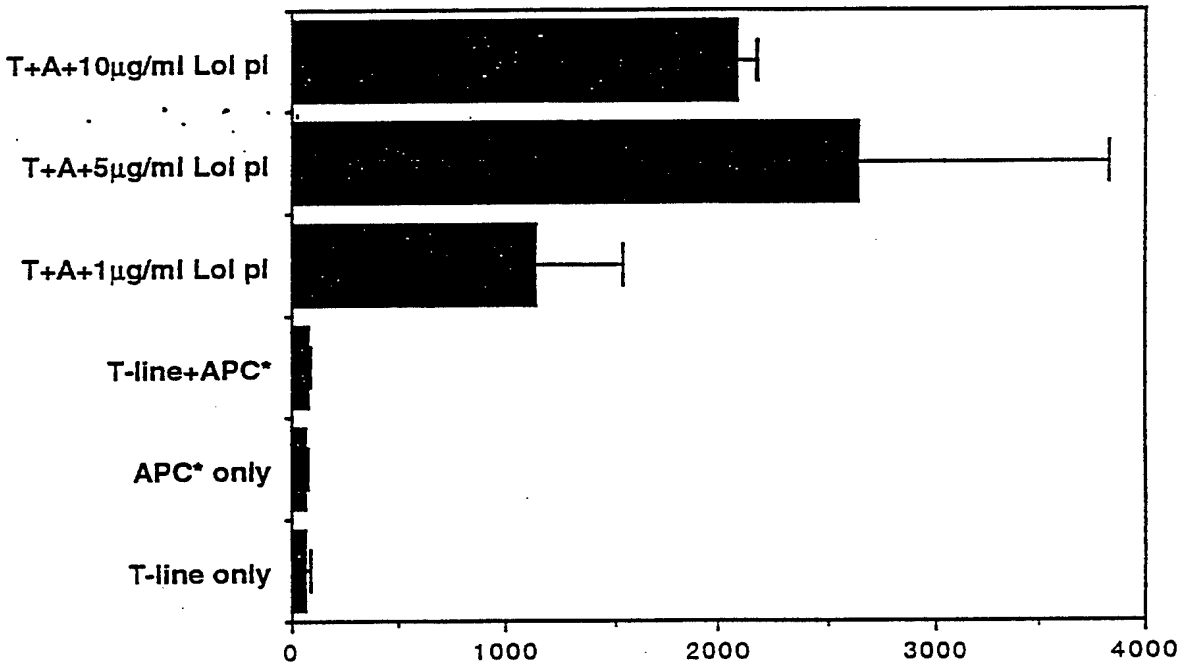


Figure 6

AS3 T cell line specificity for Lol p 1



CPM

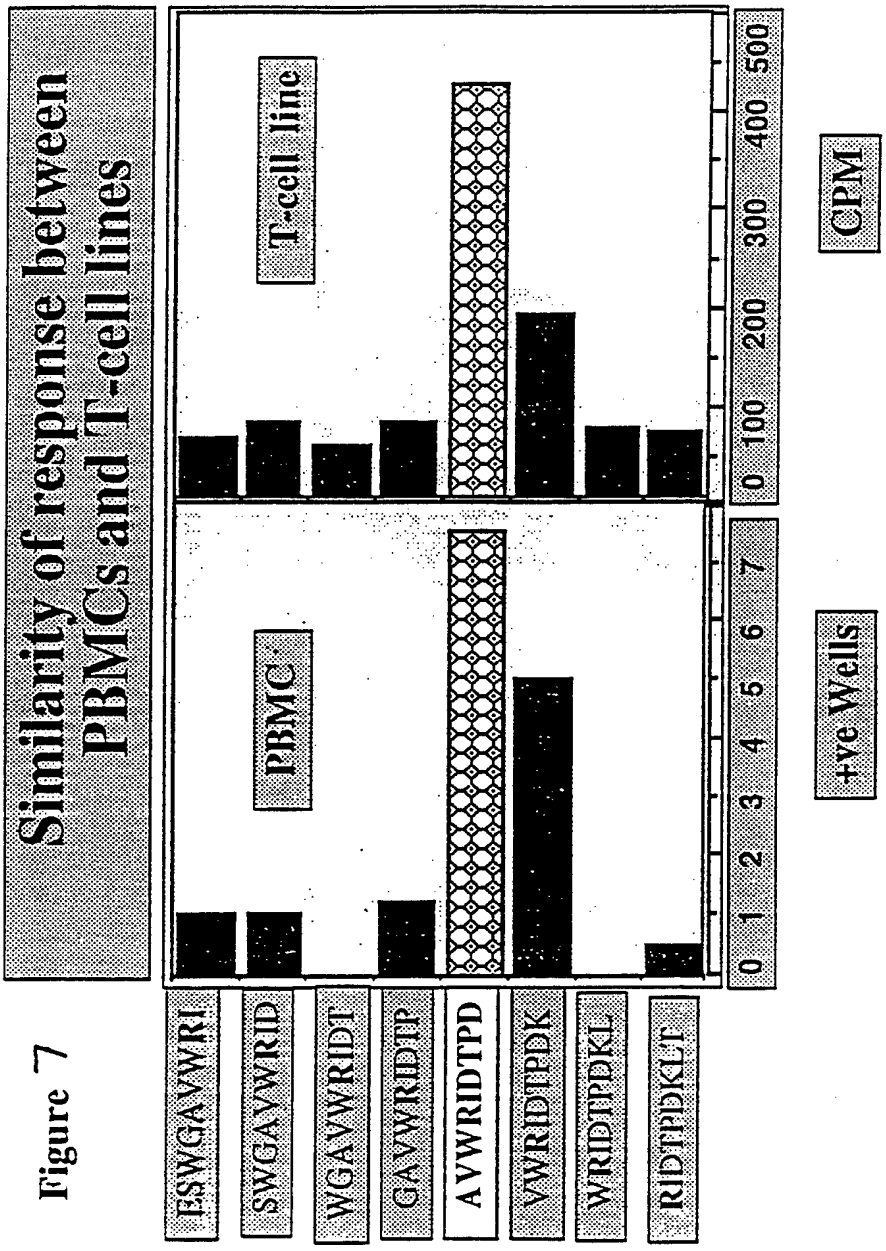

Similarity of response between PBMCs and T-cell lines
T-cell line
PBMC
ESWGAVWRI
SWGAVWRID
WGAVWRIDT
GAVWRIDTP
AVWRIDTPD
VWRIDTPDK
WRIDTPDKL
RIDTPDKLT
0 1 2 3 4 5 6 7
+ve Wells
0 100 200 300 400 500
CPM

Figure 7

FIG. 8

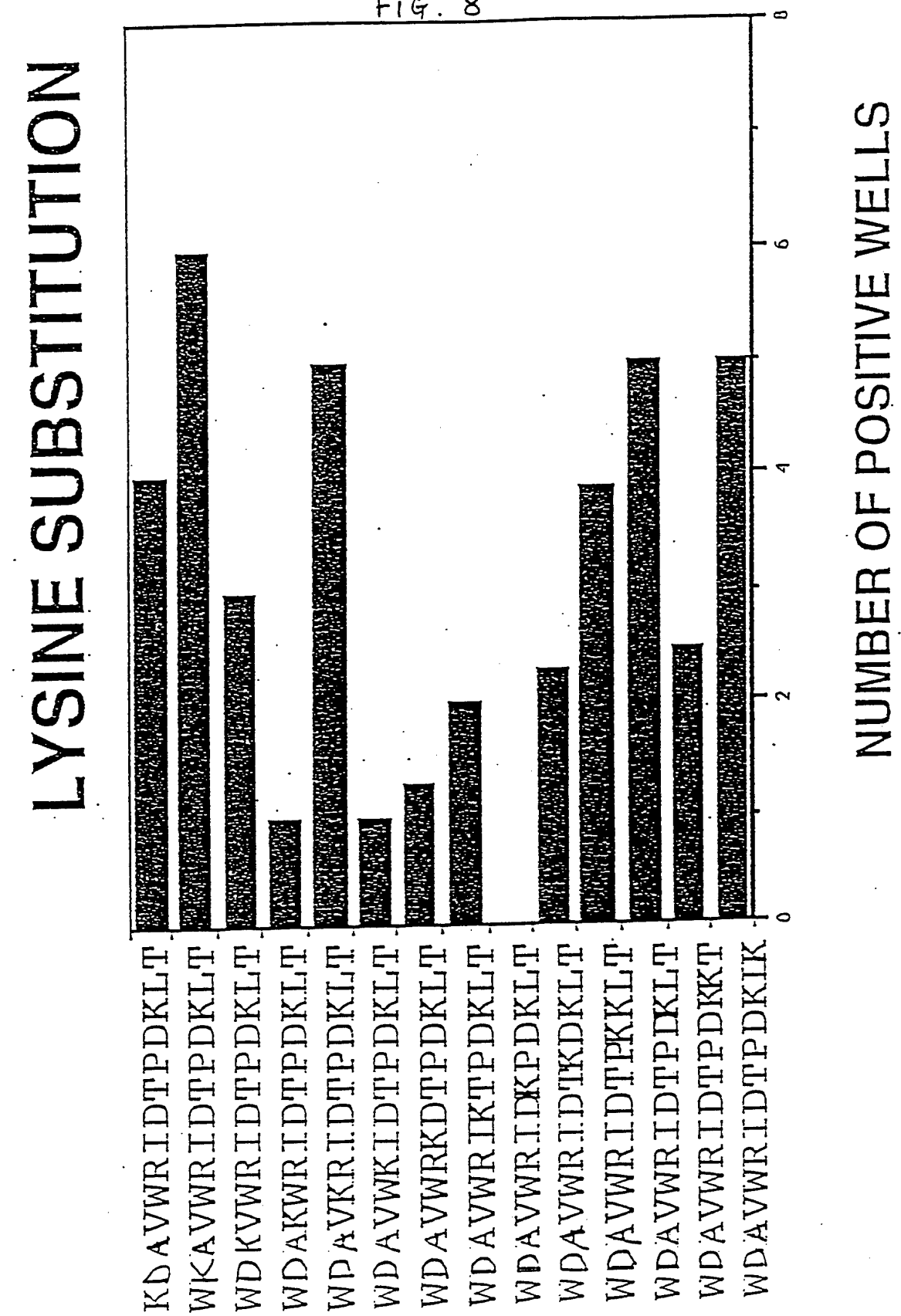
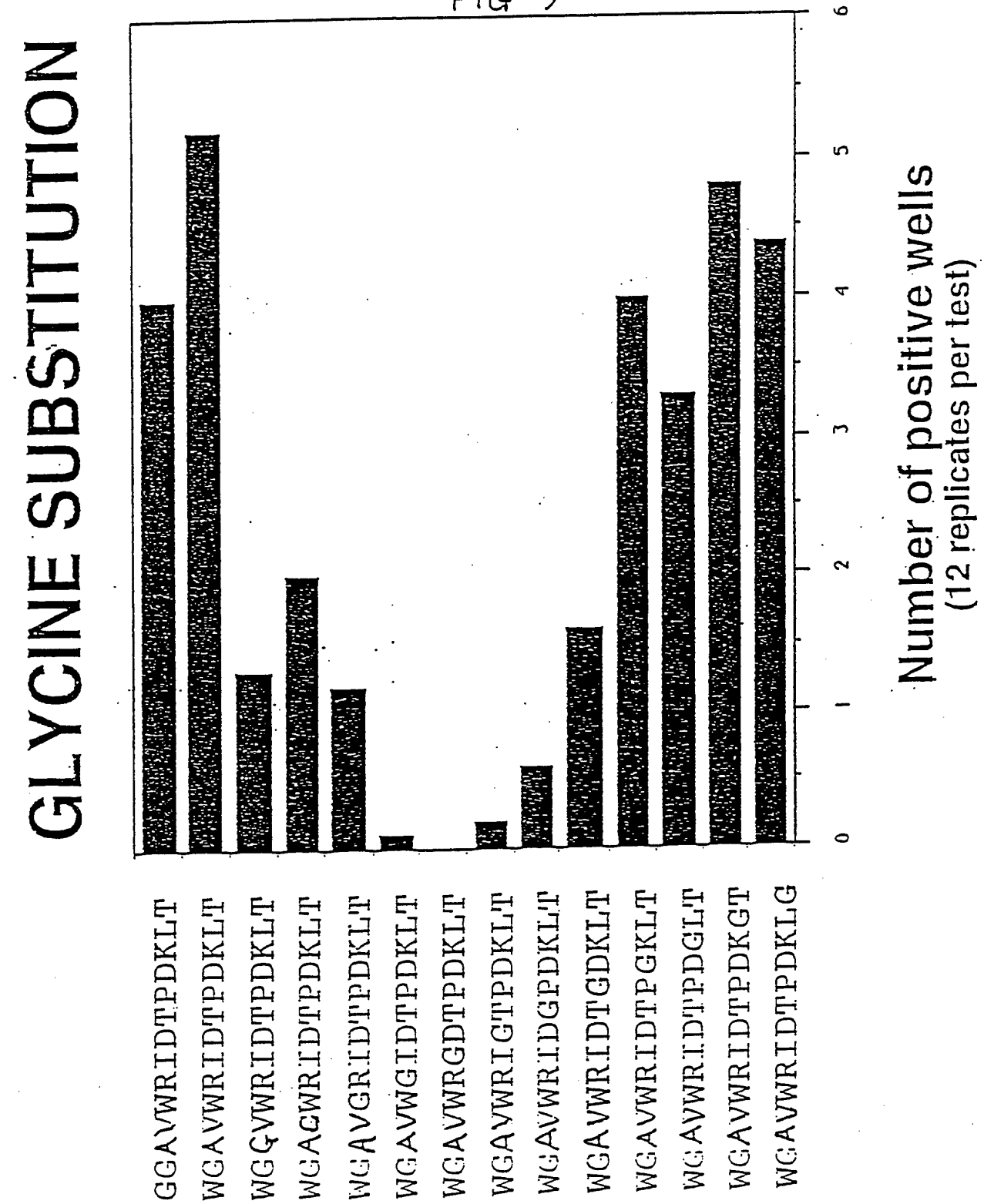

LYSINE SUBSTITUTION
KDAVWRIDTPDKLT
WKAVWRIDTPDKLT
WDKVWRIDTPDKLT
WDAKWRIDTPDKLT
WDAVKRIDTPDKLT
WDAVWKIDTPDKLT
WDAVWRKDTPDKLT
WDAVWRIKTPDKLT
WDAVWRIDKPDKLT
WDAVWRIDTKDKLT
WDAVWRIDTPKKLT
WDAVWRIDTPIKLT
WDAVWRIDTPDKKT
WDAVWRIDTPDKIK
0
2
4
6
8
NUMBER OF POSITIVE WELLS
(12 replicates per test)

FIG 9


GLYCINE SUBSTITUTION
GGAVWRIDTPDKLT
WGAVWRIDTPDKLT
WGGVWRIDTPDKLT
WGAGWRIDTPDKLT
WGAVGRIDTPDKLT
WGAVWGIDTPDKLT
WGAVWRGDTPDKLT
WGAVWRIGTPDKLT
WGAVWRIDGPDKLT
WGAVWRIDTGDKLT
WGAVWRIDTPGKLT
WGAVWRIDTPDGLT
WGAVWRIDTPDKGT
WGAVWRIDTPDKLG
0
1
2
3
4
5
6
Number of positive wells
(12 replicates per test)

INTERNATIONAL SEARCH REPORT

Interna l Application No
PCT/GB 95/01493

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K7/06 A61K38/08 //A61K39/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLINICAL AND EXPERIMENTAL IMMUNOLOGY, vol. 94, no. 1, October 1993 OXFORD,GB, pages 111-116, BUNGY ET AL. 'T cell epitopes of the major fraction of rye grass Lolium perenne (Lol p I) defined using overlapping peptides in vitro and in vivo. I. Isoallergen clone1A' cited in the application see page 114, left column, paragraph 3; figure 4 --- -/--	3,4,10, 11,13

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
6 October 1995	09. 11. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cupido, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 265, no. 27, 25 September 1990 MD US, pages 16210-16215, PEREZ ET AL. 'cDNA cloning and immunological characterization of the rye grass allergen Lol p I' see paragraph bridging pages 16213 and 16214 ---	3,4,10, 11
A	EP,A,0 403 312 (NATIONAL RESEARCH DEVELOPMENT CORPORATION) 19 December 1990 cited in the application see claims 1,7 -----	5-9

1

INTERNATIONAL SEARCH REPORT

I. ..national application No.
PCT/GB95/01493

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 12 is directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internatic Application No
PCT/GB 95/01493

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0403312	19-12-90	AT-T-	122401	15-05-95
		AU-B-	646808	10-03-94
		AU-B-	5814890	08-01-91
		CA-A-	2057860	16-12-90
		DE-D-	69019348	14-06-95
		DE-T-	69019348	21-09-95
		EP-A-	0477231	01-04-92
		WO-A-	9015878	27-12-90
		JP-T-	4505922	15-10-92